*REMARKS*

**Claim Amendment**

Claims 30-32 have been canceled by this Amendment.

Applicants submit that none of these amendments constitute new matter, and their entry is requested.

**The Present Invention**

Claim 1 is directed to an amplification-based method for producing a mammalian promoter-containing siRNA expression cassette. The method comprises (i) treating one strand of a double-stranded mammalian promoter sequence, in an amplification reaction mixture, with an oligonucleotide primer complementary to the 5' end of the mammalian promoter sequence, wherein the mammalian promoter sequence is capable of transcribing an siRNA molecule in mammalian cells. The method also comprises (ii) treating the other strand of the mammalian promoter sequence, in the amplification reaction mixture, with a second oligonucleotide primer which is complementary to the 3' end of the mammalian promoter sequence, wherein the second primer comprises a sequence which is complementary to a sequence encoding either a sense sequence of an siRNA molecule or an antisense sequence of an siRNA molecule, along with a terminator sequence. The method further comprises treating the amplification reaction mixture of steps (i) and (ii) in an amplification reaction at a temperature for annealing and extending said primers on the mammalian promoter sequence and at a temperature for denaturing the extension products to provide an amplified product comprising the mammalian promoter, a sequence encoding either the sense sequence of the siRNA molecule or the antisense sequence of the siRNA molecule, and the terminator sequence, and wherein steps (i)-(iii) are repeated a sufficient number of times to amplify the mammalian promoter-containing siRNA expression cassette. It is clear from the language of claim 1 that the primer sequences are complementary to the promoter sequence and are not complementary to the siRNA sense or antisense sequences.

**Rejection under 35 U.S.C. § 103(a)**

The Examiner rejected claims 1-9, 17, 19-23 and 30-32 under 35 U.S.C. §103(a) as being unpatentable over Shi et al. (US 2003/0180756) in view of Medina et al. (*Nucl Acids Res* **27**:1698-1708, 1999) and Dietz et al. (US 5,814,500). Applicants traverse this rejection.

The Examiner cites Shi et al. for teaching a method of producing siRNA molecules in a mammalian cell *in vitro* by preparing an expression vector comprising a mammalian RNA pol III promoter. A double stranded nucleic acid sequence encoding an siRNA molecule and a transcription termination sequence are inserted into the expression vector at a restriction site. The Examiner further cites Shi et al. for teaching that transfection of the expression vector results in efficient siRNA mediated target gene silencing in cultured mammalian cells. The Examiner cites Medina et al. for teaching a method of generating an expression vector comprising a T7 promoter operably linked to a ribozyme by using a PCR based amplification method. The Examiner contends that Medina teaches using a 5' primer for amplifying the promoter sequence and the 5' half of the ribozyme and a 3' primer for amplifying the 3' half of the ribozyme, in which the primers also include restriction sites. The Examiner cites Dietz for teaching a U1 promoter containing expression vector. The Examiner then contends that it would have been obvious to make a mammalian promoter linked siRNA vector of Shi et al. by replacing the ligation/cloning methodology of Shi et al. with the PCR based amplification methodology of Medina et al. Applicants submit that the Examiner is in error in this rejection.

Shi et al. discloses an expression cassette that comprises in the 5' to 3' direction, a pol III promoter, a first target sequence, a spacer sequence, a second target sequence complementary to the first target sequence and a pol III termination sequence. See, paragraph 68. The expression cassette may contain restriction sites for the insertion of the target sequences, such as siRNA strands. See, paragraph 14. The expression vector of Shi et al. already contains the promoter and it is the siRNA strands that are inserted into it. Thus, Shi et al. discloses preparing cassettes by cloning. Shi et al. does not disclose the PCR amplification of a pol III promoter using a single pair of primers, in which one of the primers also includes an siRNA sequence. Thus, Shi et al. does not disclose or suggest all of the elements of the claimed subject matter.

Applicants submit that Medina et al. does not cure the deficiency of Shi et al. Medina et al. discloses an overlap PCR-based approach for preparing T7-$tRNA_3^{Lys}$-ribozyme molecule. According to Medina et al., the T7-$tRNA_3^{Lys}$-ribozyme molecule is amplified using several pair of primers. One primer pair amplifies the T7 promoter sequence and the 5' half of the $tRNA_3^{Lys}$ to produce a first amplified product. See Figure 2. One of the primers in this pair is a mixture of 11 primers binding upstream of the anticodon of the $tRNA_3^{Lys}$. See "Construction" paragraph in left column on page 1699. A second primer pair amplifies the 3' half of the $tRNA_3^{Lys}$ to produce a second amplified product. See Figure 2. One of the primers in this pair is a mixture of 11 primers binding downstream of the anticodon of the $tRNA_3^{Lys}$. See "Construction" paragraph in left column on page 1699. A third primer pair amplifies an HIV-1 env coding sequence to produce a third amplified product. See Figure 2. The second and third amplified products were used as overlapping templates and were amplified using a fourth primer pair to produce a fourth amplified product. See Figure 2. The fourth primer pair consists of one of the third primers and a primer which includes the ribozyme catalytic domain and flanking sequences to the cleavage site. See "Construction" paragraph in left column on page 1699. The first and fourth amplified products were used as overlapping templates and were amplified using a fifth primer pair to produce a fifth amplified product. See Figure 2. The fifth primer pair consists of one of the third third primers and one of the first primers. This fifth amplified product is then cloned into a vector. It is evident from this description that Medina et al. does not disclose the amplification of a promoter using a primer pair as set forth in the claims, i.e., a primer complementary to the 5' end of the promoter and a primer complementary to the 3' end of the promoter. Medina et al. also does not disclose the use of a primer complementary to the 3' end of the promoter which also includes an siRNA sequence. Thus, Applicants submit that Medina et al. does not disclose all of the elements of the claimed subject matter.

Since neither Shi et al. nor Medina et al. disclose or suggest all of the elements of the claimed subject matter, the combination of Shi et al. and Medina et al. cannot and does not disclose or suggest all of the elements of the claimed subject matter. Thus, Applicants submit that the combination of Shi et al. and Medina et al. does not render the claimed subject matter

obvious. In addition, Applicants note that Dietz et al. is cited to show pol III promoters. Dietz et al. does not show an amplification process as claimed. Thus, Applicants submit that the combination of Shi et al., Medina et al. and Dietz et al. does not render the claimed subject matter obvious.

Furthermore, the expression vector of Shi et al. already contains the promoter, and it is the siRNA strands that are inserted into the expression vector under control of the promoter. Applicants submit that there is no reason to amplify a promoter in which one of the primers contains an siRNA sequence to insert the promoter-siRNA molecule into the vector of Shi et al. as the Examiner has proposed. In addition, Applicants note that Medina et al. does not disclose the method steps on which the primers are directed to the 5' and 3' ends of the T7 polymerase as detailed above. Thus, even if the PCR based amplification method of Medina et al. could be used to make the system of Shi et al., there is nothing to suggest doing so using the primers as set forth in the claimed subject matter. Applicants submit that the limitations with respect to the primers of the claimed subject matter are not suggested by the cited prior art and certainly not suggested by reference to prior art that refers to a broad PCR based overlap amplification. Thus, Applicants submit that the combination of references fails to suggests the claimed method and therefore, fails to render the claimed subject matter obvious.

In view of the above amendments and remarks, Applicants submit that the present invention is not rendered obvious by the combination of Shi et al., Medina et al. and Dietz et al. Withdrawal of this rejection is requested.

**Rejection under 35 U.S.C. § 103(a)**

The Examiner rejected claims 30-32 under 35 U.S.C. §103(a) as being unpatentable over Engelke et al. (US 2003/0148519) in view of Caplan et al. (US 2003/0149113) and Kreutzer et al. (US 2004/0001811). Applicants traverse this rejection. However, solely in the interest of expediting prosecution of the instant application and without acceding to the correctness of the rejection, claims 30-32 have been canceled which renders this rejection moot. Withdrawal of this rejection is requested.

**Conclusion**

In view of the above amendments and remarks, it is believed that the claims satisfy the requirements of the patent statutes and reconsideration of the instant application and early notice of allowance are requested. The Examiner is invited to telephone the undersigned if it is deemed to expedite allowance of the application.

Respectfully submitted,

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